File

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Mountain-Prairie Region

IN REPLY REFER TO:

MAILING ADDRESS:
Post Office Box 25486
Denver Federal Center
Denver, Colorado 80225-0486

STREET LOCATION:
134 Union Blvd.
Lakewood, Colorado 80228-1807

FWS/R6
ES

APR 26 2005

Memorandum

To: See Distribution List

From: Regional Chief, National Wildlife refuge System, Region 6 /s/ John F. Eguaras
Assistant Regional Director, Ecological Services, Region 6 /s/ Mary G. Henry

Subject: Abnormal Amphibian Assessment on Refuges 2005

The Fish and Wildlife Service is pursuing a sixth year of nationwide assessment for abnormal amphibians on National Wildlife Refuges. We would like to take this opportunity to thank both Refuge and Ecological Services Project Leaders and staff who have agreed to participate in the Fiscal Year 2005 amphibian assessments and those who have participated in the past assessments. The purpose of the assessments are to determine if abnormal amphibians occur on Service lands, develop a database on abnormal amphibians found and land use surrounding selected Refuges, and contribute to the overall knowledge of Regional and National distribution of the phenomenon. Surveys focus on newly metamorphosed frogs and require a minimum of three people for capturing metamorphs and collecting the necessary data.

We have identified the Ecological Services and Refuge stations we would like to have participate this fiscal year in the amphibian assessment (see distribution list). Dollars will be distributed to the Ecological Services field offices participating in the surveys and each Refuge will charge to a cost code provided by the respective ES field office. Based on last year's survey efforts, we have estimated that Refuge costs associated with the survey will be about $2,000 and ES costs about $6,000. Please maintain records on how funds are distributed between salary, travel, equipment, supplies, and miscellaneous.

Attached is the Region 6 summary report for refuges assessed in 2004. Results of abnormal frogs that were sent in for parasitology examination and radiography are pending, but will be sent out as soon as they are completed. A National report of amphibian abnormalities found on refuges for the first five years (2000-2004) should be available in late 2006.

It is essential that efforts between appropriate Refuge staff and the Environmental Contaminant Specialists are coordinated with regard to logistics, timing of surveys, protocols, data collection, funding, and other details. We appreciate all the interest and cooperation on this project. If you have any questions, please contact Larry Gamble in the Denver Regional Office at 303-236-4260 or Kim Dickerson in the Wyoming Field Office at 307-772-2374, extension 30.

Attachment

Distribution List

Refuges

Lostwood NWR (Karen Smith)

Arapaho NWR (Ann Timberman)

Alamosa NWR (Michael Blenden)

Rainwater Basin NWR (Gene Mack)

Mortenson NWR
(Ann Timberman, Arapaho NWR)

ES Field Offices

Bismarck, ND (Kevin Johnson)

Lakewood, CO (Laura Coppock)

Lakewood, CO (Laura Coppock)

Grand Island, NE (Christina Lydick)

Cheyenne, WY (Kim Dickerson)

DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE
REGION 6

ENVIRONMENTAL CONTAMINANTS PROGRAM

ON-REFUGE INVESTIGATIONS SUB-ACTIVITY

FINAL REPORT: YEAR 5

Nationwide Malformed Amphibian Monitoring Project

Project ID: 1130-3AMP
C:\Documents and Settings\DickersonK.IFW6-DICKERSON\My Documents\Data\Amphibian\2004rpts\R6nlrpt04.doc

compiled by
Kimberly Dickerson
Fish and Wildlife Biologist
Wyoming Field Office

for

Roxanna Hinzman, National Amphibian Coordinator
Division of Environmental Contaminants - Region 9
Washington, D.C.

February 2005

Executive Summary

In 2004, U.S. Fish and Wildlife Service (Service) personnel monitored five National Wildlife Refuges (NWRs) in Region 6, which included Alamosa NWR and Arapaho NWR in Colorado, Rainwater Basin NWR in Nebraska, Lostwood NWR in North Dakota, and Mortenson NWR in Wyoming, for the presence of abnormal amphibians. This was the first year of monitoring at each of these refuges.

Overall, there were few abnormalities found in amphibians. The refuge with the highest number of abnormalities in metamorphs observed was Lostwood NWR in North Dakota. At one site on this refuge, 5 of 31 western chorus frog (P*seudacris triseriata*) metamorphs appeared abnormal. At a second site, 7 of 52 western chorus frog metamorphs appeared abnormal. At the Rainwater Basin NWR in Nebraska abnormal metamorphs were found at five sites. The abnormalities appeared in western chorus frogs, an unknown toad species (*Bufo* sp.), and the plains leopard frog (*Rana blairi*). Two refuges in Colorado were monitored for abnormal amphibians. Western chorus frog and Woodhouse's toad (*Bufo woodhousii*) metamorphs were examined at Alamosa NWR but no abnormalities were found. At Arapaho NWR, two of the northern leopard frog (*Rana pipiens*) metamorphs and one of the western chorus frog metamorphs examined exhibited abnormalities at Arapaho NWR. At Mortenson NWR in Wyoming, four chorus frog metamorphs were found to have abnormalities.

The abnormal specimens found during the monitoring effort were sent to the University of Wisconsin for parasitological examinations as some parasites may be responsible for certain malformations. This was the second year that amphibians were examined for parasites. Results of the examinations are pending. Upon completion of the parasitological examinations, the metamorphs were preserved so that radiographs could be taken.

Table of Contents

Tables and Figures

Attached Appendices

Appendix A1. Monitoring report for Alamosa and Arapaho NWRs, site map, photos, site characterization forms, and data collection forms for Alamosa NWR, Colorado.

Appendix A2. Site map, photos, site characterization forms, data collection forms, and abnormality forms for Arapaho NWR, Colorado.

Appendix B. Monitoring report, photos, site characterization forms, data collection forms, and abnormality forms for Rainwater Basin NWR, Nebraska.

Appendix C. Monitoring report, site map, photos, site characterization forms, data collection forms, and abnormality forms for Lostwood NWR, North Dakota.

Appendix D. Monitoring report, photos, site characterization forms, data collection forms, and abnormality forms for Mortenson NWR, Wyoming.

INTRODUCTION

In recent years, an increasing number of frogs have exhibited severe abnormalities. Because of the apparent increase in amphibian abnormalities and reports that such amphibians appear on Federal Lands, the Department of Interior distributed funding to the U.S. Fish and Wildlife Service (Service) to confirm the presence of abnormal frogs on National Wildlife Refuges (NWRs).

The purpose of the monitoring effort is to: determine if abnormal amphibians occur on Service lands; develop a database on land use surrounding selected Refuges; and contribute to the overall knowledge of regional and national distribution of the phenomenon. Monitoring focuses on examining 50 - 100 newly metamorphosed frogs, preferably *Rana* species, for abnormalities at two or more sites on a refuge. A newly metamorphosed species of frog is defined as having a snout-vent length (SVL) of less than 50 mm and completing Gosner stage 46 (Gosner 1960). The effort typically requires a minimum of three people for capturing etamorphs and collecting the necessary data. At sites where *Rana* species are not present in numbers necessary to complete the monitoring, data was collected on other species of frogs (*Hyla* sp.) and toads (*Bufo* sp.). Any abnormal amphibians collected during monitoring in 2000 - 2002 were photographed, preserved, and sent to the U.S. Geological Survey National Wildlife Health Center (NWHC) in Madison, Wisconsin, for further examination. In 2003 and 2004, abnormal amphibians collected during monitoring were sent alive to the University of Wisconsin at La Crosse for parasitology examinations.

Six refuges were selected for monitoring in the year 2000 and included: Lake Alice, North Dakota; Lake Andes and Sand Lake, South Dakota; Quivira, Kansas; Ouray, Utah; and Cokeville Meadows, Wyoming. Monitoring occurred at these same refuges in 2001 except for Lake Alice NWR where water levels were too high. Monitoring occurred instead at Long Lake NWR in North Dakota. Two additional refuges were monitored in 2001 (Valentine NWR in Nebraska and Lee Metcalf NWR in Montana). In 2002, those refuges that were monitored both in 2000 and 2001 and where the occurrence of abnormal frogs was <3% were not selected for a third year. However, Long Lake NWR, Lee Metcalf NWR, and Valentine NWR were monitored for a second year. In addition, Waubay NWR in South Dakota and Flint Hills NWR in Kansas were monitored in 2002. In 2003, monitoring occurred at the same refuges as in 2002. All new refuges were monitored in 2004. These refuges included Mortenson Lake in Wyoming, Lostwood in North Dakota, Rainwater Basin in Nebraska, and Arapaho and Alamosa in Colorado.

During monitoring at Long Lake Wetland Management District (District) at Long Lake NWR in 2002 and 2003, Service personnel followed the development of metamorphosing northern leopard frogs (*Rana pipiens*) by keeping them in captivity until metamorphosis was complete. The impetus for observing the development of these metamorphs came from the District's 2001 monitoring efforts. In 2001, seven northern leopard frog metamorphs with fully developed mouths and retained tails ranging from 16-42 mm in length were collected. According to the Service's 2001 Frog Sampling Protocol "any tail >2 mm in length is considered retained if the frog's mouth is fully developed," and retained tails are considered abnormalities/malformations.

These seven frogs were recorded as abnormal/malformed, euthanized, and preserved according to protocol. Subsequently, the District staff's curiosity was piqued regarding these "tailed metamorphs."

In 2002 and 2003, District staff investigated whether frogs having a mouth developed beyond their eye (Gosner stage #45) and having a retained tail >2 mm in length are abnormal/malformed, or simply a product of developmental variability (i.e., some frogs resorb their tail at a different rate than others). District staff decided all metamorphs with tails that were captured during the 2002 and 2003 monitoring efforts and that met the above criteria would be kept in captivity to monitor their continued development. This project allowed us to see if, given adequate time, frogs captured during the late stages of metamorphosis (i.e., the mouth is developed beyond the eye) and still having a tail >2 mm in length, would eventually resorb their tail. In 2002 and 2003, all frogs captured for this project did resorb their tails. Consequently, the presence of a retained tail was no longer considered an abnormality.

SAMPLING LOCATIONS

The Mountain Prairie Region (Region 6) of the Service contains eight states (Figure 1.). Detailed sampling location information of the five refuges monitored in 2004 is provided in Table 1. Site maps and photographs of sampling locations are included in the Appendices.

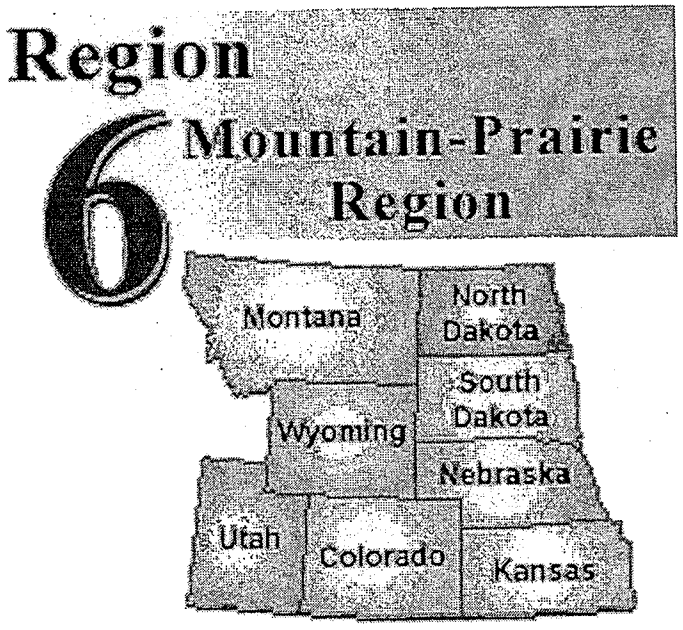

Figure 1. Mountain-Prairie Region of the U.S. Fish and Wildlife Service.

Colorado
Alamosa National Wildlife Refuge

Alamosa NWR is located in the San Luis Valley, approximately 4 miles east of the town of Alamosa, Colorado, in the southern part of the state. Alamosa NWR covers 11,169 acres and lies within the Rio Grande River floodplain. The elevation is 7,600 feet. In a region receiving an average of only 7 inches of precipitation per year, water is the lifeblood of the valley. The wet meadows, river oxbows, and riparian corridors support high wildlife diversity including songbirds, water birds, raptors, deer, beaver, and coyotes. Water resources on the refuge are managed to enhance wildlife habitat through an extensive system of ditches, water control structures, dikes, and levees. Land use surrounding the refuge includes cattle grazing, agriculture, and lands administered by the Bureau of Land Management (BLM).

The San Luis Valley has seven species of amphibians: striped chorus frog (*Pseudacris triseriata*), northern leopard frog, bullfrog (*Rana catesbeiana*), Great Plains toad (*Bufo cognatus*), Woodhouse's toad (*Bufo woodhousii*), plains spadefoot (*Spea bombifrons*), and tiger salamander (*Ambystoma tigrinum*).

Four sites were observed for metamorphs at this refuge. Site ALM01 is an oxbow/slough habitat type, characterized as a palustrine emergent semipermanently flooded wetland (USFWS 1992a). The dominant cover species is spikerush (*Eleocharis* sp.); Baltic rush (*Juncus balticus*) is also present. The immediate surrounding land use is "water body/no riparian corridor." Site ALM02 is an oxbow/slough habitat type, characterized as a palustrine emergent semipermanently flooded wetland (USFWS 1992b). The dominant cover species include spikerush, giant reed (*Phragmites autralis*), and bulrush (*Scirpus* sp.). Water milfoil (*Myriophyllum spicatum*) is present in the ponded water.

The third site (ALM03) is an oxbow/slough habitat type, characterized as a palustrine scrub-shrub wetland (USFWS 1992c), however, the dominant cover species are spikerush and giant reed grass. Floating-leaved pondweed (*Potamogeton natans*) and mare's tail (*Hippuris vulgaris*) are also present. The fourth site (ALM04) is an oxbow/slough habitat type, characterized as a palustrine emergent seasonally flooded wetland (USFWS 1992a). The dominant cover plants are spikerush, Baltic rush, sedges (*Carex* spp.), and cattail (*Typha* sp.). Water milfoil is also present. No egg masses or tadpoles were observed

Arapaho National Wildlife Refuge

Arapaho National Wildlife Refuge lies within an intermountain glacial basin known locally as North Park. The refuge is located one mile south of the town of Walden, Colorado, in the northern part of the state. Arapaho NWR covers 24,804 acres and supports diverse wildlife habitats including sagebrush-grassland uplands, grassland meadows, willow riparian areas, wetlands, and mixed conifer and aspen woodlands. The elevation ranges from 8,100 to 8,700 feet. Average annual precipitation is 12 inches per year. Arapaho NWR was established in 1967 to provide suitable nesting and rearing habitat for migratory birds. Numerous wetlands have been developed or enhanced on the refuge, fed by irrigation water from the Illinois River. The Illinois River, a tributary to the North Platte River, traverses from south to north through the eastern portion of the refuge. The refuge provides habitat for a variety of wildlife species including 198 species of birds, 32 species of mammals, 1 reptile and 5 amphibian species, and 9 species of fish. Land use surrounding the refuge includes cattle grazing, hay production, and lands administered by the BLM.

Amphibian species that occur in North Park are: striped chorus frog, northern leopard frog, wood frog (*Rana sylvatica*), western toad (*Bufo boreas*), and barred tiger salamander (*Ambystoma tigrinum mavortium*).

ARP01 is an oxbow/slough habitat type, characterized as a palustrine emergent semipermanently flooded wetland. Vegetative cover is relatively lush at this site. The dominant cover species is Nebraska sedge (*Carex nebraskensis*); forbs include bluebell (*Mertensia ciliata*), lupine (*Lupinus*

argenteus), spearment (*Mentha spicata*), curly dock (*Rumex* sp.), false Solomon's seal (*Smilacina stellata*), and elephantella (*Pedicularis groenlandica*). Willow (*Salix* sp.) shrubs are scattered throughout the site, and become dense toward the east side of the site along the Illinois River. The surrounding land uses include riparian corridor, on- and off-refuge cattle grazing, and hay production. Adult leopard frogs and chorus frogs were observed at this site. No egg masses were observed. During the last field mobilization (late August), 26 leopard frog metamorphs were captured, processed, and released at ARP01; all were normal.

ARP02 is a man-made pond habitat type characterized as a palustrine aquatic bed. The surrounding wetland is palustrine emergent. The dominant cover species is Nebraska sedge. Pondweed (*Potamogeton* sp.) occurs in the pond. The surrounding land use is on-refuge grazing. At times, cattle had to be strongly encouraged to leave the site so that the surveys could be conducted. Adult chorus frogs were observed at this site. During the first field mobilization in mid July, chorus frog tadpoles were plentiful, but were not far enough along in development for processing (most had forelimb development, but no hind limb development). During the second mobilization in late July, 63 chorus frog metamorphs were captured, processed, and released at the site. All were normal with the exception of one metamorph with a left dorsal protrusion, and one crushed metamorph. Both were believed to have been stepped on by cattle. Diagnostics were not performed. Chorus frog egg masses were observed on the east side of the pond.

ARP09 is a spring-fed man-made pond, characterized as a palustrine aquatic bed. The surrounding wetland is palustrine emergent. The dominant cover species is Nebraska sedge. Mare's tail, and water milfoil are also present. During the second field mobilization in late July, an adult northern leopard frog was captured and released. This site was visited again in late August when 4 leopard frog metamorphs were captured, processed, and released. Of the 4 metamorphs, 2 appeared normal; one was thin and lethargic with a swollen digit on the left forelimb and a small black mark on the back; one had a clubbed digit on the right forelimb and a clubbed digit on the left forelimb. Diagnostics were not performed.

Nebraska

The Rainwater Basin (RWB) area in south-central Nebraska encompasses 17 counties, covers more than 4,200 square miles, and lies within the Central Mixed-Grass Prairie ecoregion within the U.S. Fish and Wildlife Service's (Service) Platte/Kansas Rivers Ecosystem. Prior to large-scale conversion to agricultural use, there were nearly 4,000 major wetlands totaling nearly 300,000 acres in the RWB area. To date, over 90% of these wetlands have been lost with less than 400 remaining. These wetlands have historically been, and currently are, important stopover and feeding locations within the Central Flyway for waterfowl and other migratory birds. Recognizing this fact, the Service created the RWB Wetland Management District (WMD) in 1963 to protect, restore, and manage wetlands and prairie grassland habitat along with native flora and fauna, including threatened and endangered species, waterfowl, and other migratory birds. Today, the RWB WMD manages more than 24,000 acres of land on 62 different properties (primarily consisting of Waterfowl Production Areas or WPAs).

This year was the fifth year of drought for much of Nebraska. Given the high probability of some WPAs drying up before the end of summer, several potential sites were examined and monitored for tadpoles at the beginning of the field season. These sites consisted of the following 12 WPAs (with study site identification numbers in parentheses): Glenvil (RWB01), Harvard (RWB02), Mallard Haven – kiosk/cattle pond (RWB03), Mallard Haven – SW isolated wetland (RWB04), Theesen (RWB05), Smith (RWB06), Schuck – road ditch (RWB07), Schuck – pond (RWB08), Massie (RWB09), Moger (RWB10), Springer (RWB11), and Kenesaw (RWB12). The majority of these WPAs have current agriculture as a land use surrounding ≥50% of their boundaries. Corn is the primary agriculture crop. However, some fields are planted in a corn/soybean crop rotation. The primary contaminants of concern are pesticides (current use), fertilizers, and sediments.

Only four WPA locations had sufficient numbers of amphibians to be considered a study site for 2004. These four sites were Glenvil (RWB01), Harvard (RWB02), Mallard Haven – kiosk/cattle pond (RWB03), and Theesen (RWB05). The remaining eight locations were either dry, too difficult to sample due to site characteristics, and/or did not have enough tadpoles and newly metamorphosed frogs to warrant sampling.

Glenvil WPA (RWB01) is an emergent wetland with current agriculture use immediately to the north and west of the WPA. There is grassland (categorized as "other" according to the land use categories in the Standard Operating Procedures or SOPs) immediately to the east and south with current agriculture after the grassland area. Contaminant threats and concerns from surrounding land use are considered to be moderate for this WPA.

The southwest moist soil unit (MSU) of Harvard WPA (RWB02) is an emergent wetland with current agriculture use immediately to the north and west. There is grassland (categorized as "other") followed by current agriculture to the south. East of the SW MSU are more wetlands of the WPA (again, categorized as "other" according to the land use categories in the SOPs). This WPA is considered to be moderately threatened by contaminants from surrounding sources.

The Mallard Haven WPA – kiosk/cattle pond site is an emergent wetland. The RWB WMD periodically grazes cattle on different WPAs as a common management tool to restore and maintain the emergent wetland habitat. Cattle were free to graze on surrounding grassland and wetland habitat and were free to walk through the kiosk wetland site. Therefore, this site had cattle manure inputs for this summer, but cattle manure (and nutrients) are not continuous, year-round contaminant concerns for the WPA. The overall, long-term surrounding land use is wetlands (categorized as "other") to the north and west. There is a gravel access road and grassland (categorized as "other") to the east, and current agriculture to the south of the WPA. Despite the presence of cattle in the site location, Mallard Haven, as a whole, is considered to be lightly threatened by contaminants from surrounding sources, and the least contaminated site sampled this year.

Theesen WPA is an emergent wetland. This site may be the most contaminated among the four sites sampled this year. There is current agriculture to the north and east, grassland (categorized as "other") to the west, and a cattle confined animal feeding operation (CAFO) to the south.

When frogs were collected from this site, a recent rain event had caused some flooding of the road that separates the WPA from the cattle CAFO to the south. It was apparent that runoff from the CAFO could flow from the feedlot, across the road, and into the WPA.

North Dakota

Located on the Missouri Coteau (48° 37' N; 102°27' W), Lostwood NWR is a 109-km^2 area characterized by rolling hills of mixed-grass prairie and scattered aspen (*Populous tremuloides*) groves interspersed with a diversity of wetlands (Butcher 2003). Based on surface water samples taken in 2003, the U.S. Geological Survey documented pronounced levels of methyl mercury on the refuge (Johnson *et al.* 2003). Consequently, Lostwood NWR is now the locale for a 3-year study examining the trophic movement of mercury among various taxa (macro-invertebrates, amphibians, birds). Additionally, the study is examining the relationship between methyl mercury production in wetland landscapes containing prescribed burns versus unburned habitats. Since the early 1970s, prescribed burns have been conducted on Lostwood NWR to control woody vegetation and promote growth of native grasses (U.S. Fish and Wildlife Service 1998, Madden *et al.* 1999). However, burning is believed to exacerbate methyl mercury production in susceptible habitat types (Johnson *et al.* 2003). Monitoring of frogs for abnormalities at Lostwood NWR occurred in wetlands where prescribed burns occurred as well as in unburned habitats.

Three anuran species are known to occur on Lostwood NWR including the western (striped) chorus frog, northern leopard frog, and wood frog (Murphy and Danley *In Review*). Because chorus frogs are the most abundant species, data was collected on the metamorphs three sites. Site LST01 was a 0.51 ha seasonal wetland located in the wilderness area of the refuge. The wetland was surrounded by pole-size aspen and willow thickets and heavily vegetated with emergent vegetation. At the time of the survey, water depth of LST01was mostly shallow (10 – 13 cm). However, 2 deeper pools of open water (0.5 – 1.0 m) occurred in the middle of the wetland, and it was in these pools where sampling was conducted. LST01 was burned 6 times since 1982, with the last burn occurring May 2000 (Lostwood NWR, Prescribed Burn Records). In 2004, water samples were taken from LST01 and analyzed for total mercury and methyl mercury content; results from these samples are forthcoming.

Site LST02 was a 4.41 ha, semi-permanent wetland located in the control region of the refuge (-102.47440 48.61113) where prescribed burns have not taken place. The wetland was heavily vegetated with a diverse array of submergent and emergent vegetation and was surrounded by open mixed grass prairie. Water samples taken from LST02 in 2003 yielded total mercury and methyl mercury levels of 1.96 and 1.07 ng/liter, respectively.

Site LST03 was a 0.58 ha seasonal wetland found in the Kruse Management Unit of the refuge. The wetland was characterized as having uniform shallow water (≤0.3 m) and heavily vegetated with emergent vegetation (*Carex spp.*). Scattered around the wetland were aspen trees and shrubs, the majority of which appeared dead from the most recent burn in October 2003. Since 1986, this area has been burned a total of 5 times (Lostwood NWR, Prescribed Burn Records).

Water samples taken from LST03 in 2003 yielded total mercury and methyl mercury levels of 14.91 and 8.17 ng/liter, respectively.

Wyoming

Mortenson NWR was established in May 1993 to protect the last known breeding population of the endangered Wyoming toad (*Bufo baxteri*). The refuge encompasses 719 hectares of the Laramie Plains and is located approximately 24 km southwest of Laramie, Wyoming in Albany County. The refuge is currently closed to all public access to minimize impacts to the Wyoming toads. The refuge contains four impoundments including Mortenson Lake, Garber Lake, Gibbs Pond, and Soda Lake. The majority of amphibians, including the Wyoming toad, tiger salamander, and the western (striped) chorus frog, occur around Mortenson Lake because the other lakes are very alkaline. The northern leopard frog, formerly present in the Laramie Plains Basin, has been extirpated with no known reason. Mortenson Lake has an area of 32 ha and a well-developed wetland complex of bull rush (*Scirpus acutus*) and Baltic rush. Prescribed grazing is the primary land management tool. Mosquitoes are controlled on the refuge by *Bacillus thuringensis* var. *israelensis* (Bti) while the surrounding private property is sprayed with malathion.

Privately owned grasslands used for cattle grazing are located adjacent Mortenson Lake on the west and north. Land immediately south of the refuge is also grassland with a private home to the southwest. A dirt road and an adjacent lake with and emergent wetland fringe abut the refuge on the east side. The lake is on state land and designated for fishing. Although there are no regulations against motorized water craft, the lake is small and such watercraft use is not a frequent occurrence.

This was the first year of monitoring on the refuge. Various areas of the refuge were monitored for chorus frog tadpoles but only one wetland (MRL01), immediately north of Mortenson Lake, proved successful for collecting data on metamorphs. This site is classified as a wet meadow with shallow standing water (<30 cm). Beginning in early June of 2004, this wetland area had both chorus frog eggs and tadpoles; but, metamorphosis failed to occur in the tadpoles until mid-August, which is quite late. During most years, chorus frogs metamorphose during late June or early July but temperatures were much cooler-than-normal during the summer, which may have resulted in the delayed development. Most metamorphs were found in standing water contained in the hoof prints of grazing cattle.

Table 1. Sampling locations on the national wildlife refuges monitored for abnormal amphibians in 2004.

Refuge	State	Site Name	Sampling Season*	Potential Contaminants	Latitude	Longitude
Alamosa	CO	ALM02	1	None suspected	37.352861 °N	-105.748611 °W
Alamosa	CO	ALM03	1	None suspected	37.36225 °N	-105.763277 °W
Alamosa	CO	ALM04	1	None suspected	37.374916 °N	-105.770222 °W
Arapaho	CO	Fisherman Parking (ARP01)	1	Fertilizers, Nutrients	40.646111 °N	-106.479166 °W
Arapaho	CO	Soap Creek West (ARP02)	1	Nutrients	40.693055 °N	-106.42 °W
Arapaho	CO	Germ Pond (ARP09)	1	Nutrients	40.739166 °N	-106.57422 °W
Rainwater Basin	NE	Glenvil WPA (RWB01)	1	Pesticides (current), Fertilizers, Sediment, Gravel Road Runoff	40.47735 °N	-98.22242 °W
Rainwater Basin	NE	Harvard WPA (RWB02)	1	Pesticides (current), Fertilizers, Sediment	40.61045 °N	-98.18365 °W
Rainwater Basin	NE	Mallard Haven WPA (RWB03)	1	Pesticides (current), Fertilizers, Sediment	40.44405 °N	-97.73498 °W
Rainwater Basin	NE	Theesen WPA (RWB05)	1	Pesticides (current), Fertilizers, Sediment, Other (Feedlot)	40.51040 °N	-98.27009 °W
Lostwood	ND	LST01	1	Heavy Metals	48.63576 °N	-102.48362°W
Lostwood	ND	LST02	1	Heavy Metals, Nutrients	48.61113 °N	-102.4744°W
Lostwood	ND	LST03	1	Heavy Metals	48.58228 °N	-102.46549 °W
Mortenson	WY	Mortenson Lake (MRL01)	1	Pesticides, Nutrients	41.12641°N	-105.51046°W

* Number of years that the refuge has been sampled.

METHODS

Monitoring, sampling, and data collection were performed according to Standard Operating Procedures (SOPs) developed by Service's Chesapeake Bay Field Office and outlined in National Amphibian Malformation Monitoring on Refuges - 2001. Any variations from the standard SOPs by Service personnel conducting the amphibian monitoring are described under the appropriate state. In the years 2000 - 2002, any abnormal amphibians found were collected and sent to the National Wildlife Health Center (NWHC) in Madison, Wisconsin for examination by Dr. David Green to determine if the abnormality was the result of an injury or a developmental malformation.

In 2003 and 2004, live abnormal amphibians were sent to Dr. Dan Sutherland of the University of Wisconsin, La Crosse, for parasitology examination. Parasites, particularly the parasite ribieroiria, have been suspected of causing some abnormalities in amphibians (Murphy et al. 1987). Dr. Sutherland recommended that it was best to send ten abnormal individuals from a site for parasitology work. However, if ten abnormal individuals were not found at a site possible, then the sample was completed (for a total of ten individuals) by substituting normal amphibians. This was to give Dr. Sutherland the best indication possible of the parasite fauna at a site. It was also recommended that when collecting amphibians, any snails that were caught should also be sent alive to Dr. Sutherland. The only confirmed hosts for ribieroiria are planorbid "rams horn" snails. These snails may be a key link in elevated levels of ribieroiria in wetland areas inhabited by frogs. Upon completion of the parasitology examinations, specimens were preserved in a 10% formalin solution for future radiographic studies.

Colorado

Alamosa National Wildlife Refuge

The FWS Colorado Field Office (CFO) was assisted by Loree Harvey, a herpetologist with the BLM who lives in Alamosa. Loree was referred by Kelli Stone, Refuge Biologist, and was contracted by the refuge to assist with the 2004 amphibian surveys. Prior to the CFO mobilizing a team to conduct surveys, Loree conducted reconnaissance on the refuge to identify amphibian breeding locations, and to alert the CFO when metamorphs were ready to survey.

Site Characterization forms were filled out for four sites at Alamosa NWR (ALM01, ALM02, ALM03, and ALM04), and surveys were conducted by teams of four to eight members. The four sites were selected because either calling was heard or tadpoles were observed during the reconnaissance. Surveys consisted of walking the site (no systematic pattern of traversing the site was used) and looking for metamorphs. Nets (heavy duty dip nets from Ben Meadows) were used to dip into any standing water to look for tadpoles. Metamorphs were predominantly found on pond or wetland margins where soil was still very moist. Metamorphs were captured by placing either a net or a cupped hand over the metamorph, then placing it in an open gallon-size ziplock-type bag containing a small amount of water and vegetation. Metamorphs were transferred from ziplock bags to a 5-gallon bucket or a small cooler until they could be processed. Blue ice packs, 2 to 3 inches of pond water, and plenty of native vegetation were

placed in the bucket or cooler, which was partially submerged in pond water in an attempt to keep cool during collection. Capturing was conducted both during the day and at night; day and night capturing were about equally productive. Headlamps and flashlights were used at night. Metamorphs captured during the first day were held overnight and processed the following morning in the hotel room. Metamorphs captured the second day were processed that afternoon. Metamorphs were released to the same sites from which they were captured. Surveys took place during one field mobilization spanning two days.

Arapaho National Wildlife Refuge

The FWS Colorado Field Office (CFO) was assisted by Pam Johnson, Refuge Biologist, and two seasonal biologists (Crystal Bechaver and Wendy Mulherin). The refuge staff conducted reconnaissance on the refuge to identify amphibian breeding locations and to alert the CFO when metamorphs were ready to survey.

Site Characterization forms were filled out for 15 sites at Arapaho NWR (ARP01 through ARP15). Surveys were conducted by teams of two to five members. The sites that were surveyed were selected if, during the reconnaissance, calling was heard, frogs (tadpoles or adults) were observed, or suitable breeding habitat was present. Surveys consisted of walking the site (no systematic pattern of traversing the site was used) and looking for metamorphs. Nets (heavy duty dip nets from Ben Meadows) were used to dip into standing water to look for tadpoles. Metamorphs were predominantly found along pond or wetland margins where soil was still very moist, or within very small pools of water. Metamorphs were captured by placing either a net or a cupped hand over the metamorph, then placing it in an open gallon-sized ziplock-type bag containing a small amount of water and vegetation. Metamorphs were transferred to either a 5-gallon bucket or a small cooler until they could be processed. Blue ice packs, 2 to 3 inches of pond water, and plenty of native vegetation were placed in the bucket or cooler, which was partially submerged in pond water to keep cool during collection. Capturing was conducted during the daylight hours. Captured metamorphs were held in collection buckets until processing at either the hotel or onsite. Metamorphs were released to the same sites from which they were captured. Surveys took place during three separate field mobilizations, each of which spanned a period of two days. Only the sites where metamorphs were captured, processed, and released are included in the discussion below.

Nebraska

Frogs were captured according to the Capture Protocol Standard Operating Procedure (SOP) with one modification. Once a frog was captured, it was placed in a large plastic ziploc bag along with a small amount of site water and/or some wet vegetation instead of being placed in a plastic tupperware container. The ziploc bag was used because it was easier to keep track of and the top portion could be placed in a pocket (with the bottom of the bag hanging out) where it was immediately accessible. Tupperware containers were not used because they were too big to stay tucked inside a set of hip or chest waders. Therefore, the tupperware container had to be placed on the ground and was not always immediately on hand. Additionally, using ziploc bags ensured that no frogs were injured by getting caught under a lid. Overall, the ziploc bags worked very

well and did not have any observable adverse effects on the frogs. After several frogs had been collected, they were transferred to a bucket kept in the shade as described in the SOP. Data collection was conducted according to the SOP. Decontamination of field equipment occurred at the field office after all sites had been sampled according to the decontamination SOP. Used cleaning materials were disposed of at the field office. Abnormal frogs were shipped live to Dr. Dan Sutherland according to the Live Shipment SOP.

North Dakota

During the summer of 2004, wetlands in Lostwood NWR (2 sites with prescribed burns, 1 unburned site) where adult Northern leopard frogs (n = 4) had been seen were periodically sampled for tadpoles. Leopard frogs were initially chosen for the survey to allow comparisons to be made with the 2001 – 2003 malformation surveys conducted in North Dakota. However, other wetlands on the refuge also were sampled to locate tadpoles of any species. The primary method of sampling was dip-netting, although a few of the wetlands also were seined for tadpoles. Additionally, early in the season during evening hours, we listened for the chorusing of adult frogs to aid in locating wetlands for the survey. Gosner stages (Gosner 1960) of tadpoles captured in dip nets were characterized to estimate time that metamorphs would emerge from the wetlands and determine collection dates. Upon emergence, frogs were collected and inspected for malformations.

Frogs were examined using data collection guidelines developed for the National Amphibian Malformation Monitoring on Refuges survey. We measured body (snout-to-vent length) and tail (if present) length, and characterized Gosner stage of captured frogs. We inspected the animals for overall body symmetry and then systematically examined the head, torso, limbs, toes, webbing and tail (if present) of each animal. Any abnormalities were recorded, and potentially malformed frogs were shipped alive to the University of Wisconsin, LaCrosse for further analyses. Healthy frogs were released back into the wetlands in which they were captured.

Wyoming

Monitoring for chorus frog eggs and tadpoles began on June 8, 2004 and was repeated approximately weekly until metamorphosis. It was very difficult to collect metamorphs this year due to their low numbers. We found metamorphs by crawling on our hands and knees with the majority of froglet being found in standing water within cattle hoof prints. Frogs were captured by hand and placed in a large plastic container along with a small amount of site water and some wet vegetation. After collecting 50 frogs, data collection was conducted according to the SOP. Decontamination of field equipment occurred at the refuge after the site was sampled according to the decontamination SOP. Used cleaning materials were disposed of at the Wyoming Field office in Cheyenne. Abnormal frogs were also transported back to the field office and packaged according to the Live Shipment SOP. Metamorphs were shipped overnight to Dr. Dan Sutherland for parasitological examination.

RESULTS

Colorado

Alamosa National Wildlife Refuge

At ALM01, chorus frog tadpoles were observed onsite during the reconnaissance, but after a four-person team surveyed for about an hour, no egg masses, tadpoles or metamorphs were found. One adult Woodhouse's toad female with a possible extra digit on the left forelimb was captured, photographed, and released onsite.

No egg masses or tadpoles were observed at site ALM02. Three adult Woodhouse's toads were observed at the site. Fifty-one chorus frog metamorphs were captured (about half during the day and half at night). The metamorphs were held overnight and processed the following morning in the hotel room; all were normal. No egg masses were observed at site ALM03. Only three chorus frog metamorphs were captured and processed; all were normal.

At site ALM04 no egg masses or tadpoles were observed. Thirty-nine chorus frog metamorphs and 68 Woodhouse's toad metamorphs were captured, processed, and released within a period of 3 to 4 hours. All were normal (showed normal development). Unfortunately, however, 19 of the 68 Woodhouse's toad metamorphs died in holding.

Arapaho National Wildlife Refuge

Adult leopard frogs and chorus frogs were observed at site ARP01. No egg masses were observed. During the last field mobilization (late August), 26 leopard frog metamorphs were captured, processed, and released; all were normal.

Adult chorus frogs were observed at site ARP02 and during the first field mobilization in mid-July, chorus frog tadpoles were plentiful, but were not far enough along in development for processing (most had forelimb development, but no hind limb development). During the second mobilization in late July, 63 chorus frog metamorphs were captured, processed, and released at the site. All were normal with the exception of one metamorph with a left dorsal protrusion, and one crushed metamorph. Diagnostics were not performed. Chorus frog egg masses were also observed on the east side of the pond during this time.

During the second field mobilization in late July, an adult northern leopard frog was captured and released at site ARP09. This site was visited again in late August when four leopard frog metamorphs were captured, processed, and released. Of the four metamorphs, two appeared normal; one was thin and lethargic with a swollen digit on the left forelimb and a small black mark on the back; one had a clubbed digit on the right forelimb and a clubbed digit on the left forelimb. Abnormal data sheets were completed on the two metamorphs.

Nebraska

Harvard WPA was initially sampled during late morning on June 15, 2004. Harvard was sampled again, along with Glenvil WPA, on July 6, 2004. Mallard Haven WPA was sampled on July 7, 2004. Annual sampling finished with Theesen WPA on the morning of July 15, 2004. Seventy-one plains leopard frogs (*Rana blairi*) and 84 western chorus frogs were captured at Glenvil WPA. All frogs were in the process of final metmorphosis, with the plains leopard frogs ranging from Gosner stages 42-45 and the western chorus frogs ranging from stages 42-46 (Gosner 1960). The only abnormal frog captured was a western chorus frog (#56) that was missing its left hind foot. At the time of capture, the base of the foot was red and inflamed and there was a small bit of bone sticking out.

A total of 52 plains leopard frogs and 112 unknown species of toad (*Bufo* sp.) were captured at Harvard WPA. Individuals for both species ranged from Gosner stages 42-45. The toads were most likely Woodhouse's toads. They had an unspotted belly that was whitish to yellowish, had elongate parotoid glands, had cranial crests, and had dark dorsal spots that contained one red wart. They did not have a boss on the snout. These are characteristics typical of a Woodhouse's toad (Conant and Collins 1998). However, Woodhouse's toads also are reported to have a light middorsal stripe. None of the toads captured had a middorsal stripe evident. One large, adult Woodhouse's toad was captured at Harvard WPA, and the light middorsal stripe was present. It could be possible that the middorsal stripe does not appear until the toads are older than new metamorphs. Since this is not known for certain, the toads were categorized as an unknown toad species on the field data sheets and in this report. None of the plains leopard frogs were abnormal, but 5 of the toad species were. The first toad (#26) was missing its right hind calf and foot. The end of the thigh did not appear red or inflamed. The second toad (#27) was missing most of its left front limb. Only a portion of bone (less than half) for the upper part of the limb was sticking out from the body (with no flesh around the bone). No bleeding or inflammation was noticed. The third toad (#43) had all of its body parts present, but the right front limb did not move properly. The arm could be moved to face forward, but when the toad hopped, the limb moved (and stayed) facing backwards towards the rear of the toad. There did appear to be red in the area of the elbow as if there was bleeding under the skin. The fourth toad (#52) was missing approximately 70% of the left hind thigh. The remaining portion of the thigh ended at a red stump with a short length of bone sticking out of it. The last toad (#76) was missing a digit from the right front limb. The rest of the digits and foot looked red and the toad seemed reluctant to move.

Fifty plains leopard frogs were captured at the Mallard Haven WPA kiosk/cattle pond site with Gosner stages ranging from 42-46. Seven western chorus frogs and four unknown toad species were captured incidentally; all individuals were at Gosner stage 45 or 46. The toads captured at this site were identical in appearance to the toads captured at Harvard WPA. Therefore, these toads also are most likely Woodhouse's toads. Two plains leopard frogs and one western chorus frog were abnormal. The first leopard frog (#5) only had ¾ of its left hind thigh present. The second leopard frog (#20) had all of its limbs present, but the entire right hind leg was contused and red. There were no broken bones protruding from the limb. The chorus frog (#5) only had ½ of its left hind calf present. The SW isolated wetland at Mallard Haven was sampled, but only

two plains leopard frogs were captured. Both frogs were normal. One frog was at Gosner stage 45, and the other was at stage 46.

Fifty-one plains leopard frogs with Gosner stages ranging from 43-46 were captured at Theesen WPA. Nine northern leopard frogs at Gosner stages 45-46 also were captured. It should be noted that plains and northern leopard frogs are very similar in appearance with the primary distinction being two uninterrupted dorsolateral folds for the northern leopard frog versus two dorsolateral folds that are interrupted near the groin of the animal for the plains leopard frog (Conant and Collins 1998). The two species can hybridize in areas where their ranges overlap. Therefore, distinctions between the two species can be subjective. Several of the frogs captured at this site exhibited hybrid characteristics, namely one dorsolateral ridge was uninterrupted all the way to the groin but the other dorsolateral ridge was interrupted. Additionally, distance between the two interrupted lines of the interrupted ridge varied. For this study, a frog was identified as a northern leopard frog when there were two uninterrupted dorsolateral ridges or one ridge was just slightly interrupted (i.e., the two interrupted lines were very close together). All other frogs were identified as plains leopard frogs. Three plains leopard frogs were abnormal. The first frog (#3) had poor muscle development in both hind legs, and the skin of the stomach was very transparent with internal organs appearing very dark. Additionally, both eyes were completely dark so that there was no distinction between the pupil and the normal white part of the eye. The second plains leopard frog (#26) had poor muscle development in both hind legs. Additionally, there appeared to be a fluid-filled sac just above the anus. The sac was transparent and pinkish from the fluid inside. The final frog (#50) had poor muscle development for both of its front and hind limbs.

North Dakota

Chorus frog tadpoles were captured in 5 of the 16 wetlands searched at Lostwood NWR, whereas no leopard frog tadpoles were captured on any of the wetlands surveyed. Three wetlands, LST01, LST02 and LST03, contained relatively abundant chorus frog tadpoles were chosen for the malformation survey.

We estimated time that chorus frog metamorphs would emerge from the wetlands to be late July or early August 2004, based on Gosner stages of tadpoles examined beginning mid-July. Metamorph collections were attempted on 29 and 30 July, and 2, 3, 9 and 16 August 2004. A total of 83 chorus frog metamorphs were collected. Of those, 12 (14%) had potential malformations. Malformations included truncated tail (n = 4), bifurcated tail (n=1) kinked (n = 4) or indented (n = 1) tail and missing (n = 1) or shortened (n = 1) digits. By site, 31 frog metamorphs were collected from LST01, with 5 (16%) considered potentially malformed, and 52 metamorphs were collected from LST03, of which 7 (13%) were considered potentially malformed. During the collection on 30 July, 40 additional tadpoles (7 from LST01, 17 from LST02 and 16 from LST02) were collected but subsequently released because they were not at the developmental stage (Gosner stage $\geq$42) required to evaluate for malformations. Additional collections at LST02 were attempted on 9 and 16 August but no metamorphs were collected from this site.

Wyoming

Monitoring of Mortenson Lake for chorus frogs was done weekly beginning June 8 until sampling occurred mid-day on August 16, 2004. The Wyoming toad was the only other amphibian species encountered during monitoring and sampling. Weather the day of sampling was sunny and calm with an air temperature of 23°C. The water temperature in the small pools was 18°C. Two biotechnicians from Arapaho NWR and two Wyoming Field Office biologists were able to collect 50 western chorus frogs. All frogs had just metamorphosed (Gosner stage 46) or were in the final stages of metamorphosis (Gosner stage 45). Four abnormal frogs were captured. Three of the metamorphs (#4, #14, and #31) had an abnormal right eye (an abnormal small eye, small abnormal pupil, and missing eye, respectively) and the fourth metamorph (#48) appeared as if the skin had been peeled away from its lower back.

Table 2. Number of amphibians examined and number collected with abnormalities in 2004 from National Wildlife Refuges.

Refuge	Sample Date	Site Name	Common Name	Genus species	# Collected	# Abnormal	# Malformed
Arapaho	8/25/04	Fisherman Parking (ARP01)	Northern Leopard Frog	*Rana pipiens*	26	0	NA[1]
Arapaho	7/26/04	Soap Creek West (ARP02)	Western Chorus Frog	*Pseudocris triseriata*	63	1	Abnormal specimen not collected
Arapaho	8/26/04	Germ Pond (ARP09)	Northern Leopard Frog	*Rana pipiens*	4	2	Abnormal specimens not collected
Alamosa	7/14/04	ALM02	Western Chorus Frog	*Pseudocris triseriata*	51	0	NA
Alamosa	7/14/04	ALM03	Western Chorus Frog	*Pseudocris triseriata*	3	0	NA
Alamosa	7/14/04	ALM04	Western Chorus Frog	*Pseudocris triseriata*	39	0	NA
Alamosa	7/14/04	ALM04	Woodhouse's Toad	*Bufo woodhousii woodhousii*	68	0	NA
Rainwater Basin	7/0604	Glenvil WPA (RWB01)	Plains Leopard Frog	*Rana blairi*	71	0	NA
Rainwater Basin	7/06/04	Glenvil WPA (RWB01)	Western Chorus Frog	*Pseudocris triseriata*	84	1	TBD[2]
Rainwater Basin	6/15/04	Harvard WPA (RWB02)	Unknown Toad Species	*Bufo* sp.	112	5	TBD

Table 2. cont.

Rainwater Basin	7/06/04	Harvard WPA (RWB02)	Plains Leopard Frog	*Rana blairi*	52	0	NA
Rainwater Basin	7/07/04	Mallard Haven WPA (RWB03)	Plains Leopard Frog	*Rana blairi*	50	2	TBD
Rainwater Basin	7/07/04	Mallard Haven WPA (WB03)	Western Chorus Frog	*Pseudocris triseriata*	7	1	TBD
Rainwater Basin	7/07/04	Mallard Haven WPA (RWB03)	Unknown Toad Species	*Bufo* sp.	4	0	NA
Rainwater Basin	7/15/04	Theesen WPA (RWB05)	Plains Leopard Frog	*Rana blairi*	51	3	TBD
Rainwater Basin	7/15/04	Theesen WPA (RWB05)	Northern Leopard Frog	*Rana pipiens*	9	0	NA
Lostwood	7/29/04	LST01	Western Chorus Frog	*Pseudocris triseriata*	31	5	TBD
Lostwood	7/30/04	LST03	Western Chorus Frog	*Pseudocris triseriata*	52	7	TBD
Mortenson	8/16/04	Mortenson Lake (MRL01)	Western Chorus Frog	*Pseudocris triseriata*	50	4	TBD

[1]NA = Not Applicable.
[2]TBD = To Be Determined.

DISCUSSION

Colorado

This was the first year of surveying both Alamosa and Arapaho NWRs in Colorado. Overall, surveys were successful but we found it very difficult to collect the number of metamorphs per site that is currently recommended in the SOPs because of the region is very arid.

Surveys were conducted during both the night and the day. However, we found that for chorus frogs in particular, there seemed to be no advantage to surveying during the night versus during the day. But, when surveying on hot days during the middle of the day, extra precautions need to be taken to prevent exposing the metamorphs to wide temperature variation. We believe this inadvertently occurred at Alamosa (which resulted in the death of Woodhouse's toad metamorphs).

Having people at the refuges who are available to assist in the reconnaissance and daily checking of metamorph development is invaluable. The staff from the Colorado Field Office would not have been nearly as successful in capturing and surveying metamorphs had it not been for the on-refuge staff (FWS and contract).

Alamosa National Wildlife Refuge

Woodhouse's toads and chorus frogs were the most common amphibians at Alamosa NWR. One adult toad female with a possible extra digit on the left forelimb was captured at site ALM01 but because it was an adult, the toad was photographed and released onsite rather than being sent in for a parasitological exam and radiography. At site ALM02, chorus frog metamorphs were abundant enough for collecting abnormality data but at ALM03 only chorus frog metamorphs were captured.

Capturing metamorphs at site ALM04 was most successful as 39 chorus frog metamorphs and 68 Woodhouse's toad metamorphs were processed and released. Unfortunately, however, 19 of the 68 Woodhouse's toad metamorphs died in holding. Capturing was conducted during the heat of the day from 11:30 a.m. until 2:00 p.m. with air temperature change from 27 to 34°C during that time. It is thought that the metamorphs were held too long in the ziplock collection bags when air temperatures were quickly rising. The lesson learned is to get metamorphs as quickly as possible to a holding container where cooler temperatures can be maintained.

Arapaho National Wildlife Refuge

Fifteen sites were checked for metamorphs at Arapaho NWR but only three sites had amphibian metamorphs. Adult leopard frogs and chorus frogs were observed at site ARP01, however, it was not until late August when leopard frog metamorphs were collected. Even so, only 26 metamorphs were captured but all were normal. At ARP02, chorus frog metamorphs were abundant with data collected on 63 of them. All were normal with the exception of one

metamorph with a left dorsal protrusion, and one crushed metamorph. Both were believed to have been stepped on by cattle so diagnostics were not performed.

Site ARP09 was the only other site where metamorphs were found; but only four leopard frog metamorphs were captured. Of the four metamorphs examined, two appeared normal, one was thin and lethargic with a swollen digit on the left forelimb and a small black mark on the back, and one had a clubbed digit on the right forelimb and a clubbed digit on the left forelimb. Abnormal data sheets were completed on the two abnormal metamorphs but because only four leopard frogs were collected from this site, the specimens were not sent to for parasitological examination and radiography.

Nebraska

Upon review of the abnormal frogs collected from all four sampling locations, the western chorus frog (#56) from Glenvil, the plains leopard frog (#20) from Mallard Haven, and two of the toads (#52 and 76) from Harvard exhibited signs of current, fresh injuries. It was difficult to determine if the two of the remaining three abnormal toads from Harvard (#27 and 43) were the result of and injury just by visual observation. Although toad #27 did not have any signs of bleeding or inflammation, there was a piece of bone protruding from the limb. This is indicative of an older injury since the bone was still there, but there was no bleeding and no flesh around the bone. Toad #43 still had its entire front limb, but it did not function properly. The last abnormal toad (#26) and plains leopard frog (#5) at Harvard and Mallard Haven, respectively, along with the abnormal western chorus frog (#5) from Mallard Haven, were potentially malformed. It was impossible to determine whether or not the abnormality was the result of an old injury that had healed or a true malformation that occurred during the developmental process from egg to tadpole or tadpole to frog. Two of the plains leopard frogs (#3 and 26) from Theesen also were potentially malformed. Frog #3 had abnormal looking eyes and a dark belly with the internal organs appearing dark. This frog was kept alive in captivity for about a week; therefore, the darkness most likely was not from any internal bleeding. The cause of the dark discoloration is unknown. Frog #26 had a tumor-like sac just above the anus but underneath the tail. The sac was filled with fluid and was a pinkish red in coloration. The remaining abnormal plains leopard frog (#50) from Theesen had poor muscle development but, otherwise, had all limbs and body parts present. This may have been a symptom of malnourishment.

The commonly accepted background rate of malformation for amphibians is 3%. Although a determination as to the number of frogs with true malformations is forthcoming, given the number of potentially malformed frogs collected in this study, one site (Theesen WPA) slightly exceeded the background malformation rate when a minimum sample size of 50 individuals for a given species is used. The potential malformation rate for Theesen WPA was 3.9% for plains leopard frogs. The remaining sites with a minimum of 50 individuals per species had potential malformation rates below the background malformation rate. Glenvil WPA had a potential malformation rate of 0% for both plains leopard and western chorus frogs. Harvard WPA had a potential malformation rate of 0.9% for the unknown toad species and 0% for the plains leopard frog. Finally, the potential malformation rate at Mallard Haven WPA was 2% for the plains leopard frog.

Although Mallard Haven was considered to be the least contaminated site overall, it had the second highest potential malformation rate. It would be interesting to sample this site next year and see if the absence of cattle in the area changes the results. It is possible that potentially malformed frogs from Mallard Haven were actually just abnormal with the missing limbs being old injuries from the cattle. It also would be interesting to sample Theesen WPA again next year because it was slightly above the background malformation rate, and it would be interesting to see if Theesen exceeds the background rate a second time. If Theesen does exceed the background malformation rate for two consecutive years, there may actually be some contaminant impacts occurring on the WPA that would warrant additional investigation. Finally, it would be good to try to capture more than the minimum number of individuals for a given species at Theesen, Mallard Haven, and Harvard to see if their potential malformation rates change.

North Dakota

Based on our sampling efforts, chorus frogs were the most common frog found on Lostwood NWR in 2004, whereas Northern leopard frogs were rare. Additionally, vocalizations of chorus frogs could be heard in many of wetlands initially searched, but leopard frogs were not heard at any wetlands. Chorus frog metamorphs during our study emerged approximately 4-5 weeks later in the season than we expected (Murphy and Danley *In Review*). Therefore, it is possible that unseasonably cool weather this summer influenced survival and development of leopard frog tadpoles that tend to emerge after chorus frogs in this region. Thus, sampling in future years could yield larger numbers of leopard frog tadpoles making collections of this species possible. However, our findings on relative abundance of the 2 species were consistent with those reported by Murphy and Danley (*In Review*). Nevertheless, our collections still did not yield large numbers of metamorph chorus frogs at any of the 3 sites and this could be due to a combination of factors.

First, unseasonably cool weather during the 2 days immediately prior to the collections likely inhibited the final development of chorus frog tadpoles. This probably influenced the success of our initial collection day, when the greatest number of personnel was available to help capture metamorphs. For example, during the collections on 30 July, 58% of the captured frogs, including 100% of the 17 frogs captured at LST02, could not be evaluated for malformations because individuals had not developed to the appropriate Gosner stage (Gosner stage $\geq$42) and had to be released. In addition, the small size of the frogs in general (maximum snout to vent length 3.8 cm; Northern Prairie Wildlife Research Center Web Site: www.npwrc.usgs.gov/narcam/ idguide/chorus.htm) likely contributed to lower number of animals captured.

We were most successful capturing frogs at Gosner stages 42 and 43, when the forelimbs had emerged and tails were pronounced or only beginning to atrophy; at this stage the tadpoles were not able to hop out of the water, making it easier to capture this tiny frog. By Gosner stages 44 and 45, the frogs could hop and were able to exploit the shallow-watered, grass edges of the wetlands where dip-netting was not possible. This is likely what happened at site LST02 where zero individuals were captured on our last sampling date, 16 August. Furthermore, the low numbers of animals collected could have been a result of high predation on tadpoles by natural

predators in these small, isolated wetlands. Predators of tadpoles found at LST01 during the season included tiger salamanders, fishing spiders (Family Pisauridae) and giant water beetles (Family Belostomatidae). Tiger salamanders also were documented at sites LST02 and LST03. Finally, it is possible that egg production by adult chorus frogs and development was reduced due to high methyl mercury levels on the refuge (Johnson *et al.* 2003). Known effects of methyl mercury accumulation in tissues of aquatic organisms include poor growth and reproduction, behavioral effects, and mortality (Annual Mercury Report 1999 http://www.deq.state.la.us/surveillance/mercury/1999/introduction.htm).

Inferences cannot currently be made on the specific causes of the chorus frog malformations we observed at Lostwood NWR. It is possible some of the malformations could have been due to injuries sustained by developing tadpoles while evading capture by aquatic predators. Injuries also could have been sustained during previous dip netting surveys to monitor Gosner stages of developing tadpoles. Nevertheless, we documented a high incidence (14%) of potentially malformed chorus frogs at the 2 burned wetlands of which one wetland (LST03) had high methyl mercury levels in 2003. (Note: results are forthcoming on methyl mercury levels in water samples for the 3 sites during Spring 2004). Other known anthropogenic influences on the refuge include limited cattle grazing with potential for causing nutrient enrichment in localized wetlands. Additional survey work at Lostwood NWR would yield insights into the causes of frog malformations in this region. We recommend future malformation surveys at Lostwood NWR focus search and collection efforts on chorus frogs, rather than the Northern leopard frogs. Not only were chorus frogs more easily detected on Lostwood NWR refuge, but also, less information exists regarding malformations in this species.

Wyoming

Chorus frogs at Mortenson Lake typically lay their eggs in early June with metamorphoses completed by late June or early July. Chorus frog eggs and tadpoles were observed at Mortenson as early as June 8, 2004 but because of a cool and wet summer, metamorphosis was delayed until mid-August. Numerous chorus frogs could be heard calling from early June through July but numbers of chorus frog tadpoles were low. A conversation with an adjacent landowner on 8/13/04 revealed that frost had occurred in the area the two previous nights, which also hampered the metamorphosing of chorus frogs. We were able to locate one very shallow wet meadow area with enough metamorphs to collect data. We never observed metamorphs in other commonly used wet meadow areas. Collection of 50 metamorphs resulted in finding four abnormal frogs. Metamorph #4 had an abnormally small right eye, metamorph #14 had a pupil in the right eye that was small and not normally shaped, metamorph #31 had a right eye that was completely missing, and metamorph #48 had an area on its back where the skin appeared to be peeled back. We were unable to determine if the latter abnormality was an injury. No bleeding or fresh red tissue was exposed. Unfortunately because the metamorphs were so small, photos of the abnormalities in metamorphs, #14, #31, and #48 did not turn out.

We were disappointed that more metamorphs were not found for additional data collection because it was curious that three of the four abnormalities found were with the right eye. The commonly accepted background rate of malformations in amphibians is 3% (*Ouellet 1997;*

Hoppe 2001). Because we are unsure if the abnormality from metamorph #48 was the result of an injury or an actual malformation, the rate of malformations in chorus frogs from Mortenson Lake this year ranged from 6-8%, which is above the background rate.

CONCLUSIONS

Assessment of amphibians for abnormalities on National Wildlife Refuges was successful in 2004. Overall, there were few abnormalities found in the amphibians. Determination of whether the abnormalities are deformities, malformations, or the result of parasites or injuries is pending.

Acknowledgements

Many thanks to the Ecological Services Environmental Contaminants Biologists and the Refuge staff who made the amphibian monitoring successful. Also, the staff from the Colorado Field Office would like to thank Loree Harvey, a herpetologist with the BLM who lives in Alamosa, Colorado; Jesse Munoz, Annette Casados, and Joel Chirhart who assisted with surveys at Alamosa NWR; and, Barb Osmundson, Crystal Bechaver, Wendy Mulherin, and Pam Johnson who assisted with surveys at Arapaho NWR.

The staff from the Grand Island Ecological Services Field Office would like to thank Jeff Drahota, RWB WMD wildlife biologist, for his assistance in monitoring potential sites at multiple WPAs and collecting and measuring frogs and toads. In addition, thanks are extended to summer seasonal technicians Matt Rabbe, Joe Speer, and Kyle Graham.

The staff from the Bismarck, North Dakota gives special thanks to Brenda Curry for searching for and monitoring the development of chorus frog tadpoles. Many thanks also to Brenda Vaness, Mike Olson, Roger Collins, Bill Bicknell, Eric Schmidt, for helping out with the tadpole metamorph collections. Thanks to Refuge Manager, Will Meeks, for providing additional volunteers and field equipment. Thanks to Kevin Johnson, Bob Murphy and Gregg Knutsen for their review of this report.

The staff from the Wyoming Ecological Services Field Office would like to thank Anne Timberman, Pam Johnson, Crystal Bechaver, and Eugene Hornyak of Arapaho National Wildlife Refuge for their coordination and assistance with data collection. Many thanks are also extended to Jessica Homyack with the Wyoming Field Office for assisting in the data collection and sample shipments.

REFERENCES

Butcher, R.D. 2003. *America's National Wildlife Refuges: A Complete Guide.* Roberts Rinehart Publishers. Lanham, Maryland. 714 pp.

Conant, R, and J. Collins. 1998. *A Field Guide to Reptiles and Amphibians: Eastern and Central North America.* Houghton Mifflin Company. Boston, New York. 616 pp.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica. 16:183-190.

Hoppe, D.M. 2001. Linking malformations to amphibian declines: History of malformed anurans in Minnesota and interspecific differences in their occurrences. In: M.J. Lannoo, ed., *Status and Conservation of United States Amphibian, Volume 1: Conservation Essays.* University of California Press. Berkeley, CA.

Johnson, K.M., W. Meeks, T. Custer, and C. Custer. 2003. Trophic movement of mercury and its effects to wildlife on Lostwood National Wildlife Refuge, North Dakota. Project ID 6N52, North Dakota Ecological Services Field Office, Region 6, Department of the Interior, U.S. Fish and Wildlife Service. 18 pp.

Madden, E.M., A.J. Hansen, and R.K. Murphy. 1999. Influence of prescribed fire history on habitat and abundance of passerine birds in northern mixed-grass prairie. Canadian Field-Naturalist. 113:627-640.

Murphy, R. K. 1987. Observations of the wood frog in northwestern North Dakota. Prairie Naturalist. 19:262.

Murphy, R.K. and R.F. Danley. In Review. Amphibians and reptiles in a mixed grass prairie in northwestern North Dakota. Prairie Naturalist.

Ouellet, M., J. Bonin, J. Rodrigue, J.L. DesGranges, and S. Lair. 1997. Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. Journal of Wildlife Diseases. 33: 95-104.

U.S. Fish and Wildlife Service. 1998. Comprehensive conservation plan: Lostwood National Wildlife Refuge. U.S. Fish and Wildlife Service, Kenmare, North Dakota.

U.S. Fish and Wildlife Service (FWS). 1992a. National Wetland Inventory Map. Alamosa East, Colorado, 7.5-Minute Quadrangle.

U.S. Fish and Wildlife Service (FWS). 1992b. National Wetland Inventory Map. Lasauses, Colorado, 7.5-Minute Quadrangle.

U.S. Fish and Wildlife Service (FWS). 1992c. National Wetland Inventory Map. Pikes Stockade, Colorado. 7.5-Minute Quadrangle.

APPENDICES